DEVELOPMENT OF A MULTIPLEX PCR METHOD FOR DETECTION OF THE GENES ENCODING 16S rRNA, COAGULASE, METHICILLIN RESISTANCE AND ENTEROTOXINS IN *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

*A multiplex polymerase chain reaction (PCR) method was developed for simultaneous detection of the genes encoding methicillin resistance (*mecA*), SEs A, B and C (*sea, seb *and* sec*), coagulase (*coa*), and 16S rRNA. The primers for amplification of the 16S rRNA gene were specific for* Staphylococcus *spp., and the primers for* coa *were specific for* Staphylococcus aureus. *Based on the results, the multiplex PCR was accomplished at an optimal* Mg^{2+} *concentration of 1.0 mM and at an annealing temperature of 56C. This multiplex PCR method was performed with 71 strains of* S. aureus *and 51 strains of six other bacterial species. Among the* S. aureus *strains tested, 40.0% (28/71) were found to contain the* mecA *gene. One of the 28* $mecA^{+}$ *strains was not resistant to methicillin. The* sea, seb *and* sec *genes were present in 47.9%*

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(34/71), 5.6% (4/71) and 8.5% (6/71) of S. aureus *strains, respectively. The sensitivity of this multiplex PCR method was approximately 104.5 pg of genomic DNA per reaction, which was equivalent to an estimated* 2.4×10^3 *cfu of* S. aureus or 3.64×10^4 *copies of genome equivalent.*

PRACTICAL APPLICATIONS

The developed multiplex polymerase chain reaction (PCR) method will be a useful tool for the detection and identification of *Staphylococcus aureus* from foods, clinical samples and environmental surveys. In particular, the routine use of this multiplex PCR method for the detection of foodborne *S. aureus* could be used to monitor the presence of enterotoxins and the emergence of methicillin resistance in a population that, to date, has had a relatively low incidence of methicillin resistance.

INTRODUCTION

Staphylococcus aureus, a gram-positive bacterium, is one of the most common clinical and foodborne pathogens worldwide (Lowy 1998; Weigel *et al.* 2003; Foster 2005; Cagatay *et al.* 2007). *S. aureus* produces many important virulence factors including SEs, which are the main causes of diarrhea, vomiting and other symptoms associated with *S. aureus* infection. It was reported that more than 70% of *S. aureus* strains produced one or more enterotoxins (Jorgensen *et al.* 2005). Because of their thermal stability, SEs remain a great hazard even in heat-processed foods (Akineden *et al.* 2001). Several SEs from SEA to SEE, SEG to SEO, SEU and SEV have been characterized according to serological analysis and classification, and most of their genes have been sequenced (Akineden *et al.* 2001; Omoe *et al.* 2002; Thomas *et al.* 2006; Vimercati *et al.* 2006). Five of these SEs (SEA to SEE) are recognized as major causes of foodborne illness (Vimercati *et al.* 2006), with SEA, SEB and SEC being the most frequently implicated in foodborne outbreaks (Balaban and Rasooly 2000).

There has been an enormous increase in the isolation of MRSA strains that has been attributed to the widespread use of methicillin in clinical settings and in food animal production facilities for decades (Panlilio *et al.* 1992; Oliveira *et al.* 2002). In addition, the gene encoding methicillin resistance (*mecA*) has been widely used as a molecular marker of methicillin resistance in detection and typing of *Staphylococcus* spp. (Ubukata *et al.* 1990; Carroll *et al.* 1996; Jonas *et al.* 2002; Francois *et al.* 2003). SEs increase the competi-

tiveness of *S. aureus*, and antibiotic resistance gives *S. aureus* a greater ability to distribute widely (Massey *et al.* 2006).

Recently, many polymerase chain reaction (PCR) methods have been developed to detect enterotoxin genes or methicillin resistance-related genes of *S. aureus* (McLauchlin *et al.* 2000; Mehrotra *et al.* 2000; Jonas *et al.* 2002; Francois *et al.* 2003; Padmapriya *et al.* 2003). Some of those methods were developed to detect several *S. aureus* toxins and methicillin resistance, but the SEs and methicillin resistance genes were not amplified in the same reaction (Mehrotra *et al.* 2000). To our knowledge, the identification of both SEs and methicillin resistance genes in a single multiplex PCR has not been reported.

The 16S rRNA gene of staphylococci contains DNA sequences that are highly conserved at the genus level but are variable among other bacterial genera (Baron *et al.* 2004). Several primers designed against the 16S rRNA gene have proven useful for identification of staphylococci (Mason *et al.* 2001; Yang *et al.* 2002). The 16S rRNA gene primer pair used in this multiplex PCR method was previously shown to be conserved in 19 *Staphylococcus* species and subspecies (Zhang *et al.* 2004). In addition, the coagulase gene (*coa*) was reported as a specific target for the detection of *S. aureus* at the species level (Chiou *et al.* 2000; Karahan and Cetinkaya 2006; Sabat *et al.* 2006). Therefore, the combined detection of both the 16S rRNA and coagulase genes may be a reliable set of markers for the detection of *S. aureus*.

The aim of this study was to identify *S. aureus* strains via amplification of the genes encoding the 16S rRNA and coagulase, and to determine the presence of the methicillin resistance gene (*mecA*) and the genes encoding the most common foodborne outbreak-associated SEs (*sea*, *seb* and *sec*) in a single multiplex PCR.

MATERIALS AND METHODS

Strains and Culture Medium

A total of 135 bacterial strains (Table 1) were cultured in Luria–Bertani broth at 37C for 6 h with shaking at 250 rpm before extraction of genomic DNA. All staphylococci isolates were separated from clinic samples and were identified by the Institute of Clinical Pharmacology at Peking University using API Staph Ident (bioMerieux, Shanghai, China).

Identification of Methicillin Resistance

The methicillin resistance of *S. aureus* strains was tested by the standardized agar dilution method (Wikler *et al.* 2006). The experiments were

TABLE 1.
BACTERIAL STRAINS USED IN THIS STUDY

Bacterial species/strains	Number	Source
Staphylococcus aureus isolates	67	*
S. aureus ATCC6538	1	†
S. aureus ATCC25923	1	†
S. aureus ATCC29213	1	†
S. aureus CMCC26001	1	†
S. aureus ATCC 27940	1	‡
S. aureus ATCC 13565	1	‡
Staphylococcus haemolyticus strains	8	*
Staphylococcus epidermidis strains	7	*‡
S. saprophyticus ATCC 15305	1	‡
S. saprophyticus ATCC 43809	1	‡
S. lugdunensis ATCC 43809	1	‡
S. schleiferi ATCC 43808	1	‡
S. xylosus ATCC 29971	1	‡
S. sciuri ATCC 29062	1	‡
S. capitis ATCC 49325	1	‡
S. chromogenes 3688	1	‡
S. simulans 13044	1	‡
S. intermedius ATCC 29663	1	‡
Salmonella spp. strains	5	†
Escherichia coli strains	22	†
Listeria monocytogenes strains	4	†
Vibrio parahaemolyticus strains	6	†
Total	135	

*Clinical isolates were obtained from Peking University Health Science Center, Beijing, China.
†Bacterial strains were acquired from Bor Luh Food Safety Center, Shanghai Jiao Tong University, Shanghai, China.
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carried out at the Institute of Clinical Pharmacology at Peking University. Briefly, *S. aureus* colonies were taken from an overnight sheep blood agar plate, and a suspension was prepared in Mueller–Hinton broth equivalent to that of the 0.5 McFarland standards. Approximately 10^4 cfu of each isolate was inoculated on Mueller–Hinton agar (supplemented with 4% NaCl) containing serial twofold dilutions of methicillin (16, 8, 4, 2 or 1 μg/mL). The microdilution trays were incubated at 35C for 24 h. Strains were identified as methicillin resistant in this study if the minimal inhibitory concentration was greater than 4 μg/mL on agar plates after 24 h of incubation at 35C.

Genomic DNA Extraction

A modified cetyltrimethylammonium bromide method was used for extraction of bacterial genomic DNA (Kalia *et al.* 1999; Tang *et al.* 2006). One milliliter of an overnight culture was used to extract genomic DNA. The genomic DNA pellet was resuspended finally in 20 μL sterile water and was stored at −20C. The concentrations of the genomic DNA were determined by measuring the absorbance at 260 nm using a DU 800 UV/Visible Spectrophotometers System (Beckman, Shanghai, China).

PCR Primers

All primers in this study (Table 2) were analyzed by Premier v5.0 (PREMIER Biosoft International, Palo Alto, CA) and were synthesized by Shanghai Bio-tech Corporation (Shanghai, China).

DNA Amplification

One microliter of the bacterial DNA sample was added to 24 μL PCR mixture containing 1 U of *rTaq* DNA polymerase (Tiangen Biotech Co. Ltd., Beijing, China); 1 × Taq buffer (200 mM Tris-HCl [pH 8.4], 200 mM KCl and 100 mM $[NH_4]_2SO_4$); 1.0 mM $MgCl_2$; 0.25 mM of each deoxyribonucleotide triphosphate (Tiangen Biotech Co. Ltd.); 50 nM of each primer for the amplification of the 16S rRNA, *coa* and *mecA* genes; 100 nM of each primer for the amplification of *sea* gene; and 75 nM of each primer for the amplification of *seb* and *sec* genes. All PCR amplifications were carried out in Peltier Thermal Cycler PTC-200 (Bio-Rad Laboratories, Hercules, CA). The amplification conditions were as follows: initial denaturation at 94C for 5 min; followed by 30 cycles of 94C for 30 s, 56C for 30 s and 72C for 30 s; with a final extension at 72C for 5 min before 16C for 10 min.

Six microliters of the amplified PCR products was used for the separation by electrophoresis in a 2.0% agarose gel at 140 V in 1 × Tris–acetate–ethylenediaminetetraacetic acid buffer for 40 min and visualization with UV illumination (Shanghai Tanon Science & Technology Co. Ltd., Shanghai, China) after staining with ethidium bromide for 15 min. Standard DNA molecular weight markers (100 bp or 1 kb DNA ladder, TianWei Science & Technology Co. Ltd., Beijing, China) were included in each run.

Optimization of Amplification Conditions

Each pair of primers was used in separate amplification reactions employing the protocol described previously. Three *S. aureus* strains were used for the optimization: *S. aureus* 05B038 (*mecA*$^+$ and *sea*$^+$), *S. aureus* 05L198 (*seb*$^+$) and

TABLE 2.
PRIMER SEQUENCES USED IN THIS STUDY

Target gene	Primer	Oligonucleotide sequences (5′-3′)	GenBank accession number	Amplicon size (bp)	Reference
16S rRNA	16S-F	AACTCTGTTATTAGGGAAGAACA	NC_002758.2	756	Zhang *et al.* 2004
	16S-R	CCACCTTCCTCCGGTTTGTCACC			
coa	COA-F	CCTCAAGCAACTGAAACAACA	BA000018.3	151	This study
	COA-R	TGAATCTTGGTCTCGCTTCAT			
mecA	MECA-F	GTAGAAATGACTGAACGTCCGATAA	X52593	310	Perez-Roth *et al.* 2001
	MECA-R	CCAATTCCACATTGTTTCGGTCTAA			
sea	SEA-F	ATTAACCGAAGGTTCTGTAGA	M18970.1	552	Tang *et al.* 2006
	SEA-R	TTGCGTAAAAAGTCTGAATT			
seb	SEB-F	TGTATGTATGGAGGTGTAAC	NC_002951.2	270	Sharma *et al.* 2000
	SEB-R	ATA GTG ACG AGTTAG GTA			
sec	SEC-F	ACCAGACCCTATGCCAGATG	AB084256	371	Cremonesi *et al.* 2005
	SEC-R	TCCCATTATCAAAGTGGTTTCC			

S. aureus SA6 (*sec*$^+$). The gradient of annealing temperature ranged from 50.0 to 62.0C, and the gradient of Mg^{2+} concentration ranged from 0.5 to 2.5 mM.

Specificity and Reliability Test

Seventy-one *S. aureus* strains and 51 strains of six other bacterial species were tested at the optimal multiplex PCR condition using the genomic DNA from *S. aureus* ATCC29213 strain (*sea*$^+$) as a positive control, and double distilled water as a negative control. This multiplex PCR assay was further evaluated using 30.0 ng of the genomic DNA from the *S. aureus* strain 05B038 (*mecA*$^+$ and *sea*$^+$) in the presence of an equal amount of DNA from strains of *Salmonella*, *Escherichia coli*, *Listeria monocytogenes* or *Vibrio parahaemolyticus*.

Sensitivity Test

All target genes were examined in a series of multiplex PCRs to determine the sensitivity of the assay using the following strains: *S. aureus* 05B038 (*mecA*$^+$ and *sea*$^+$), *S. aureus* 05L198 (*seb*$^+$) and *S. aureus* SA6 (*sec*$^+$). These tests were carried out using the optimal Mg^{2+} concentration (1.0 mM) with 1 U of *rTaq* DNA polymerase at 56C. The *S. aureus* DNA samples were prepared in a 10-fold dilution, and 1 μL of each dilution was used in this multiplex PCR assay. The quantities of the genomic DNA from the *S. aureus* strain 05B038 ranged from 1.045 to 1.045×10^4 pg per reaction; the quantities of the genomic DNA from the *S. aureus* strain 05L198 ranged from 11.05 to 1.105×10^5 pg per reaction; and the quantities of the genomic DNA from the *S. aureus* SA6 ranged from 2.757 to 2.757×10^4 pg per reaction.

RESULTS

Optimization of Amplification Conditions

The primers for the amplification of the 16S rRNA, *coa*, *mecA* and *sea* genes worked well at all temperatures tested (details not shown). The annealing temperature of the multiplex PCR in this study was chosen at 56C, and the PCR amplification products from each of the target genes at 56C are shown in Fig. 1 (lanes 1–6).

When testing various Mg^{2+}concentrations, the product of the 16S rRNA gene gave a clearer and brighter band at 1.0 mM of Mg^{2+} (Fig. 2, lanes 2, 8 and 13) than at other Mg^{2+} concentrations (0.5, 1.5, 2.0 or 2.5 mM), and all the expected target genes were amplified at this concentration. Higher Mg^{2+} concentrations (more than 2.0 mM) might have inhibited the *rTaq* polymerase

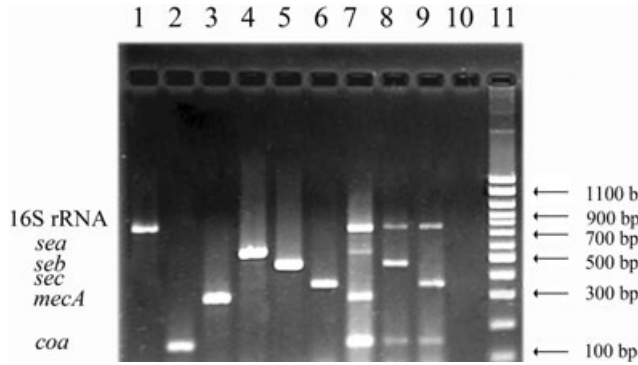

FIG. 1. SINGLEPLEX AND MULTIPLEX POLYMERASE CHAIN REACTION (PCR) ASSAYS FOR THE DETECTION OF METHICILLIN RESISTANCE AND ENTEROTOXIN GENES IN *STAPHYLOCOCCUS AUREUS* STRAINS

Lane 1, 16S rRNA amplicon from *Staphylococcus aureus* 05B038; lane 2, *coa* amplicon from *S. aureus* 05B038; lane 3, *mecA* amplicon from *S. aureus* 05B038; lane 4, *sea* amplicon from *S. aureus* 05B038; lane 5, *seb* amplicon from *S. aureus* 05L198; lane 6, *sec* amplicon from *S. aureus* SA6; lanes 7–9, multiplex PCR products from *S. aureus* strains 05B038, 05L198 and SA6, respectively; lane 10, multiplex PCR using *Escherichia coli* genomic DNA; lane 11, 100 bp DNA marker.

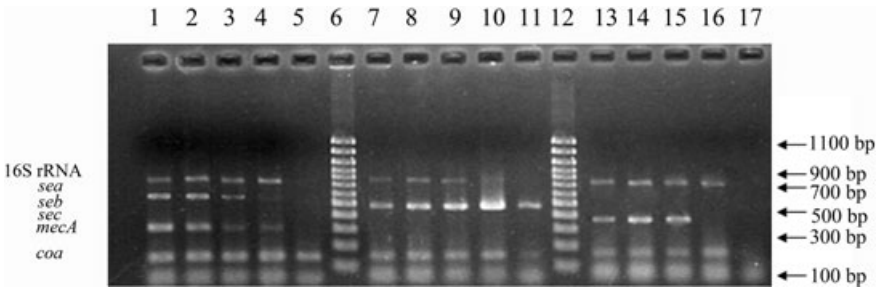

FIG. 2. EFFECT OF Mg^{2+} CONCENTRATION ON THE MULTIPLEX POLYMERASE CHAIN REACTION (PCR) ASSAY

The concentrations of Mg^{2+} were 0.5, 1.0, 1.5, 2.0 and 2.5 mM from left to right for each strain. Lanes 1–5, *Staphylococcus aureus* 05B038; lanes 7–11, *S. aureus* 05L198; lanes 13–17, *S. aureus* SA6; lanes 6 and 12, 100 bp DNA marker.

activity, resulting in a reduced amplification in multiplex PCR (Fig. 2). Therefore, 1.0 mM Mg^{2+} was used in subsequent PCR experiments. The final condition of the multiplex PCR system, as determined from the optimization experiments, was 1.0 mM Mg^{2+} at an annealing temperature of 56C for 30 cycles. As shown in Fig. 1, the tested primers of multiplex PCR worked efficiently at the chosen multiplex PCR conditions.

Specificity and Reliability Tests

A total number of 122 bacterial strains (Table 1) were tested using the multiplex PCR method. The *coa* and 16S rRNA genes were amplified from all *S. aureus* strains tested by this multiplex PCR (Table 3). To substantiate the multiplex PCR method, all *S. aureus* strains tested by the multiplex PCR were also screened for the presence of individual enterotoxin genes by singleplex PCRs. The results of the singleplex PCRs corresponded precisely with those from multiplex PCR (data not shown), demonstrating the reliability of the multiplex PCR method.

Amplicons were generated using the primers of the 16S rRNA gene, but not for the *coa* gene, when DNA from other species of *Staphylococcus* were tested using the multiplex PCR assay (Fig. 3). None of the 37 strains of other bacterial genera yielded any PCR products from any of the primer sets included in the multiplex PCR (data not shown). It is therefore concluded that the primers for amplification of the 16S rRNA gene were specific for *Staphylococcus* spp., as previously reported (Zhang *et al.* 2004). The specificity of the *coa* primers for the detection of *S. aureus* was also demonstrated by the results of the multiplex PCR tests described previously. The specificity of the *coa* primer set was further tested against additional strains of *Staphylococcus* spp. (Table 1 strains from source †) in a singleplex assay. The two additional strains of *S. aureus* yielded a *coa* PCR product, but none of the 11 other *Staphylococcus* spp., including the coagulase-positive species *Staphylococcus intermedius* and *Staphylococcus schleiferi*, yielded a *coa* amplicon (data not shown). The results of the multiplex assays (Table 3 and Fig. 3) and the singleplex PCR using the *coa* primers (data not shown) demonstrated the specificity of this primer set for *S. aureus*.

In addition, it was found from the tests by the multiplex PCR method that the *sea*, *seb* and *sec* genes were present in 47.9% (34/71), 5.6% (4/71) and 8.5% (6/71) of the tested *S. aureus* strains, respectively. In this study, 38.0% (27/71) of the tested *S. aureus* strains were both enteroxin gene(s) (*sea*, *seb* or *sec*) and *mecA* negative; 8.5% (6/71) of the *S. aureus* strains were *mecA*$^+$ and enterotoxin gene negative; and 29.6% (21/71) of the strains were *mecA*$^+$ and had one or more enterotoxin gene(s). None of the MRSA strains yielded an *seb* amplicon. There were 28 *S. aureus* strains that yielded an *mecA* amplicon, but only 27 *S. aureus* strains were phenotypically resistant to methicillin. The *mecA* gene may not be properly expressed in the *mecA*$^+$ methicillin-sensitive strain. The two strains of *Staphylococcus haemolyticus* and one strain of *Staphylococcus epidermidis* tested yielded 16S rRNA gene and *mecA* gene amplicons, but no enterotoxin genes were detected in these two *Staphylococcus* species (Fig. 3).

TABLE 3.
METHICILLIN RESISTANCE AND MULTIPLEX POLYMERASE CHAIN REACTION RESULTS FOR *STAPHYLOCOCCUS AUREUS* STRAINS

S. aureus strain	Gene amplified						Methicillin*	Total
	sea	*seb*	*sec*	*mecA*	16s	*coa*		
ATCC25923, ATCC6538, CMCC26001, 05A046, 05A075, 05A100, 05C209, 05D361, 05F104, 05G021, 05G064, 05G251, 05H150, 05I043, 05L022, 05L216, 05L228, 05N119, 05F318, 05I052, 05L201, 05E162, 05E186, 05E209, 05E024	–	–	–	–	+	+	S	27
ATCC29213, 05B085, 05A188, 05B043, 05C224, 05G226, 05H048, 05I073, 05K037, 05O261, 05O263	+	–	–	–	+	+	S	11
05D004, 05M146	+	–	+	–	+	+	S	2
05L085	+	–	+	+	+	+	S	1
05I056, 05N074, 05L198	–	+	–	–	+	+	S	3
05O114	–	+	+	–	+	+	S	1
SA6	–	–	+	–	+	+	S	1
05C212, 05C215, 05C288, 05C299, 05O073, 05Z073	–	–	–	+	+	+	R	6
05B008, 05B012, 05B033, 05B038, 05B041, 05B059, 05B086, 05B255, 05B289, 05C052, 05C077, 05C078, 05C136, 05C155, 05D131, 05F148, 05F158, 05H058, 05K241, 05P157	+	–	–	+	+	+	R	20
05D154	–	–	+	+	+	+	R	1
Totals	34	4	6	28	71	71	R = 27	

*The tests of methicillin resistance for the bacterial strains were carried out at the Institute of Clinical Pharmacology at Peking University, using the standardized agar dilution method (16, 8, 4, 2 or 1 μg/mL of methicillin, respectively).

S, methicillin sensitive; R, methicillin resistant (a minimal inhibitory concentration greater than 4 μg/mL of methicillin was considered resistant).

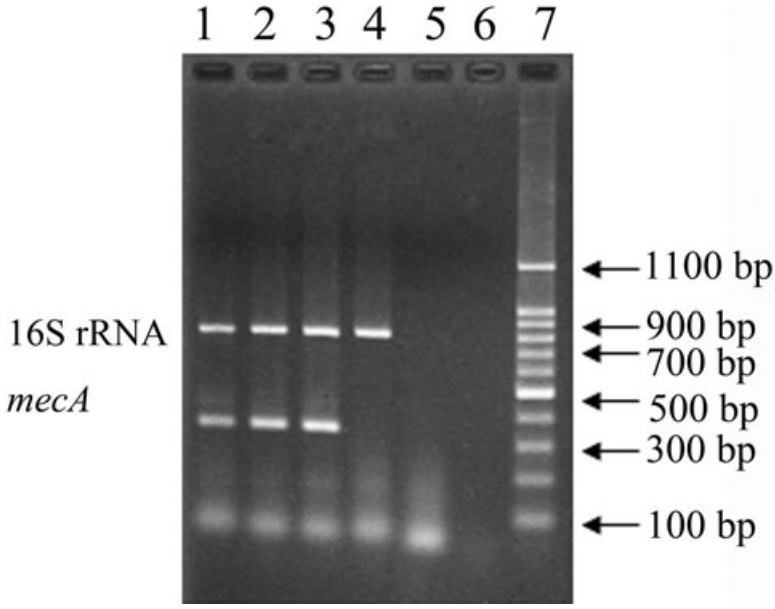

FIG. 3. MULTIPLEX POLYMERASE CHAIN REACTION (PCR) OF *STAPHYLOCOCCUS HAEMOLYTICUS* AND *STAPHYLOCOCCUS EPIDERMIDIS* STRAINS
Lane 1, *S. haemolyticus* 05G86; lane 2, *S. epidermidis* 05I078; lane 3, *S. haemolyticus* 05K203; lane 4, *S. haemolyticus* 05G218; lane 5, *Escherichia coli*; lane 6, distilled water (negative control); lane 7, 100 bp DNA marker.

The reliability of the multiplex PCR method was also tested by carrying out the PCR using *S. aureus* DNA in the presence of equal amounts of genomic DNA from other bacteria. There were no unexpected bands when *S. aureus* DNA was mixed with DNA from other bacteria including *Salmonella*, *E. coli*, *L. monocytogenes* and *V. parahaemolyticus* (data not shown). This indicated that the presence of DNA from non-staphylococci strains did not affect the accuracy of the multiplex PCR method.

Sensitivity Test

As shown in Fig. 4, the detection limit of 16S rRNA, *sea* and *mecA* genes was 104.5 pg per reaction (Fig. 4, lane 3), and the detection limit of *coa*, *seb* and *sec* genes was 10.4, 11.1 and 27.6 pg per reaction, respectively (Fig. 4, lanes 3, 11 and 18). Therefore, all the target genes were detectable at 104.5 pg per reaction by the multiplex PCR method.

In the present study, 10^8 cfu of *S. aureus* typically yielded 4.4 µg of DNA (data not shown). This result was similar to that reported by Cremonesi

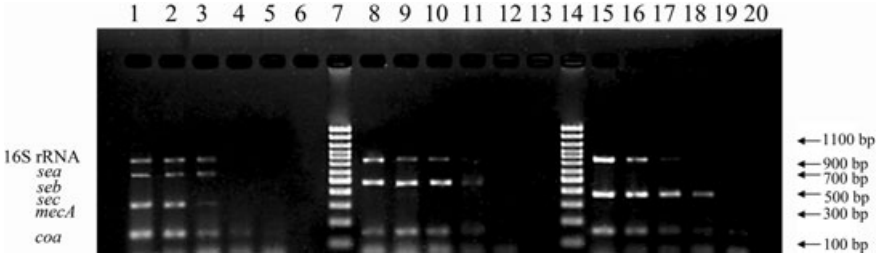

FIG. 4. SENSITIVITY OF MULTIPLEX POLYMERASE CHAIN REACTION (PCR) ASSAY
Lanes 1–5, quantities of *Staphylococcus aureus* 05B038 DNA were 1.045×10^4, 1.045×10^3, 1.045×10^2, 1.045×10 and 1.045 pg, respectively; lanes 8–12, quantities of *S. aureus* 05L198 DNA were 1.105×10^4, 1.105×10^3, 1.105×10^2, 1.105×10 and 1.105 pg; lanes 15–19, quantities of *S. aureus* SA6 DNA were 2.757×10^4, 2.757×10^3, 2.757×10^2, 2.757×10 and 2.757 pg; lanes 6, 13 and 20, distilled water (negative controls); lanes 7 and 14, 100 bp DNA marker.

et al. (2005), who obtained approximately 10 μg of DNA from 10^8 cfu of *S. aureus*. Therefore, the detection limitation can be calculated as 2.4×10^3 cfu. According to the calculation of Yang *et al.* (2002) that 2.78 fg DNA is equivalent to one *S. aureus* genome, the limit of detection of 104.5 pg of the multiplex PCR would be equivalent to 3.64×10^4 genome equivalents. It is well known that *Staphlyococcus* spp. grow in clusters of cells, and it has been reported that these clusters of 10–20 cells represent a single cfu (Wilkins *et al.* 1972; Duguid 1989). This means that one cfu of *S. aureus* may actually represent 10–20 genome equivalents. This could explain the apparent 15-fold difference in PCR sensitivity when it is expressed as cfu or genome equivalents.

DISCUSSION

An accurate multiplex PCR method was developed for detecting *S. aureus* and determining the presence of methicillin resistance and enterotoxin genes. This method was proven to be specific and reliable using 71 strains of *S. aureus* and 51 strains of six other bacterial species.

In the multiplex PCR, the detection of *S. aureus* at the genus and species levels was accomplished through the amplification of the genes encoding 16S rRNA and coagulase, respectively. As demonstrated by our multiplex PCR, the primer pair performed in the specific amplification of the 16S rRNA gene from species of *Staphylococcus* and the *coa* primers designed for this study were specific for *S. aureus* strains (Table 3).

It was found that 38.0% (27/71) of the *S. aureus* strains tested were resistant to methicillin. Other studies on clinical *S. aureus* strains detected a similar incidence of MRSA strains (Bach *et al.* 2007); however, there was a lower incidence of methicillin resistance in *S. aureus* isolated from foods (around 25%) (Nitzsche *et al.* 2007). We found a high correlation between methicillin resistance and the presence of the *mecA* gene in the *S. aureus* strains tested. All the MRSA strains yielded *mecA* amplicons, but one $mecA^{+}$ strain (*S. aureus* 05L085) was not resistant to methicillin. Furthermore, *mecA* amplicons were identified in some strains of *S. haemolyticus* and *S. epidermidis* in this work. The presence of *mecA* in species of *Staphylococcus* other than *S. aureus* was previously demonstrated (Carneiro *et al.* 2004).

It was found that 77.8% (21/27) of the MRSA strains contained one or more SE gene(s) (*sea, seb* or *sec*); however, only 43.2% (19/44) of MSSA strains contained SE gene(s). The reason for the higher incidence of SE genes in MRSA than in MSSA is not thoroughly understood. It has also been reported that the incidence of MRSA that produced SEs was greater than the incidence that did not produce SEs (Moon *et al.* 2007). The presence of virulence genes (including enterotoxin genes) and antibiotic resistance confers a competitive advantage upon *S. aureus* (Hershow *et al.* 1992; Mizobuchi *et al.* 1994), and studies indicate an increase in the isolation of toxin-containing MRSA strains (Takesue *et al.* 1993). Recent studies pointed out that the *mecA* gene is present on the mobile staphylococcal chromosomal cassette *mec* (Katayama *et al.* 2003; Jansen *et al.* 2006), and some of the enterotoxin genes, along with other virulence factors, are part of mobile pathogenicity islands (Novick 2003; Novick and Subedi 2007). Thus, enterotoxin-producing MRSA can develop via the acquisition of *mecA* by toxin-producing MSSA or via the acquisition of toxin genes by MRSA (Layer *et al.* 2006). A recent study indicated that high mobility of the *mecA* gene may be more prevalent than the movement of the enterotoxin genes (Witte *et al.* 2000).

In conclusion, a specific and reliable multiplex PCR method was developed for the detection of 16S rRNA, *coa*, *sea*, *seb*, *sec* and *mecA* genes in *S. aureus* in this study. This is the first PCR method developed for the detection of *S. aureus* that also determines the presence of the genes for methicillin resistance and the foodborne outbreak-associated enterotoxins A, B and C. When coupled with appropriate enrichment and separation procedures, this PCR method will be a useful tool for the detection and identification of *S. aureus* from foods, clinical samples and environmental surveys. In particular, the routine use of this multiplex PCR method for the detection of foodborne *S. aureus* could be used to monitor the presence of enterotoxins and the emergence of methicillin resistance in a population that, to date, has had a relatively low incidence of methicillin resistance.

NOMENCLATURE

MRSA	methicillin-resistant *S. aureus*
MSSA	methicillin-sensitive *S. aureus*
SE	staphylococcal enterotoxin

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